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The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future needs

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Abstract This paper introduces a special issue on the ecotoxicology and environmental chemistry of nanoparticles (NPs), and nanomaterials (NMs), in the journal Ecotoxicology. There are many types of NMs and the scientific community is making observations on NP ecotoxicity to inform the wider debate about the risks and benefits of these materials. Natural NPs have existed in the environment since the beginning of Earth's history, and natural sources can be found in volcanic dust, most natural waters, soils and sediments. Natural NPs are generated by a wide variety of geological and biological processes, and while there is evidence that some natural NPs can be toxic, organisms have also evolved in an environment containing natural NPs. There are concerns that natural nano-scale process could be influenced by the presence of pollution. Manufactured NPs show some complex colloid and aggregation chemistry, which is likely to be affected by particle shape, size, surface area and surface charge, as well as the adsorption properties of the material. Abiotic factors such as pH, ionic strength, water hardness and the presence of organic matter will alter aggregation chemistry; and are expected to influence toxicity. The physico-chemistry is essential to understanding of the fate and behaviour of NPs in the environment, as well as uptake and distribution within organisms, and the interactions of NPs with other pollutants. Data on biological effects show that NPs can be toxic to bacteria, algae, invertebrates and fish species, as well as mammals. However, much of the ecotoxicological data is limited to species used in regulatory testing and freshwater organism. Data on bacteria, terrestrial species, marine species and higher plants is particularly lacking. Detailed investigations of absorption, distribution, metabolism and excretion (ADME) remain to be performed on species from the major phyla, although there are some data on fish. The environmental risk assessment of NMs could be performed using the existing tiered approach and regulatory framework, but with modifications to methodology including chemical characterisation of the materials being used. There are many challenges ahead, and controversies (e.g., reference substances for ecotoxicology), but knowledge transfer from mammalian toxicology, colloid chemistry, as well as material and geological sciences, will enable ecotoxicology studies to move forward in this new multi-disciplinary field.

Keywords Nanomaterials · Ecotoxicity · Natural nanoparticles · Risk assessment

Introduction

This paper introduces a special issue on the ecotoxicology and environmental chemistry of nanoparticles (NPs), and nanomaterials (NMs), in the journal Ecotoxicology. There are many types of NMs and the scientific community is making observations on NP ecotoxicity to inform the wider debate about the risks and benefits of these materials. NMs have been defined as material with at least one dimension

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between 1 and 100 nm (Roco 2003; SCENHIR 2005; Moore 2006). However this is a somewhat arbitrary definition, and for ecotoxicology, we should also consider NMs with a distribution of particle sizes around the nanoscale that may include some primary particles larger than 100 nm; or larger aggregates of nanoparticles (NPs) of a few hundred nanometers (Handy and Shaw 2007; Handy et al. 2008). In mammalian respiratory toxicology sizes of particulate matter (PM) have been traditionally defined as coarse particles (diameter between 10 and 2.5 µm, $PM_{10-2.5}$), fine particles (2.5 µm or less, $PM_{2.5}$), or ultrafine particles (<0.1 µm, $PM_{0.1}$). Nanoparticles can therefore also be regarded as ultrafine particles, and emerging ecotoxicological data might be compared with some of the ultrafine particle exposures performed on rodents (see Handy and Shaw 2007 for a review).

Manufactured NMs are designed to achieve particular physico-chemical properties that relate to the product application. The materials can be carbon-based such as carbon spheres (e.g., Buckminster fullerenes or C_{60}, Fagan et al. 1991), carbon nanotubes (Smith et al. 2007), metal-based NPs (Federici et al. 2007), composite NMs or multi-layer NPs (e.g., platinum core–silica shell, $Pt@SiO_2$, Kim et al. 2004), or NPs with an external coating or capping agent (e.g., functionalised zinc oxide NPs, Wu et al. 2007). There are currently many types of nanoproducts on the market, and many potential benefits of these new materials. The products and applications include electronics, optics, textiles, medical devices, cosmetics, food packaging, water treatment technology, fuel cells, catalysts, biosensors and agents for environmental remediation (e.g., Roco 2003; Freitas 2005; Karnik et al. 2005; Aitken et al. 2006; Brody et al. 2006).

There is now a wider debate about the risks and benefits of the many manufactured NMs and consumer products (Royal Society 2004; US EPA 2005; Owen and Depledge 2005; Handy and Shaw 2007; Owen and Handy 2007), and this includes consideration of risks to the environment (Owen and Handy 2007). The ecotoxicology community is only at the beginning of understanding the potential risks to wildlife associated with manufactured NMs. Some of the key knowledge gaps and challenges are summarised (Fig. 1). We recognise that these materials may have unusual physico-chemical properties, or behaviours in water (e.g., colloid chemistry), that are less familiar to

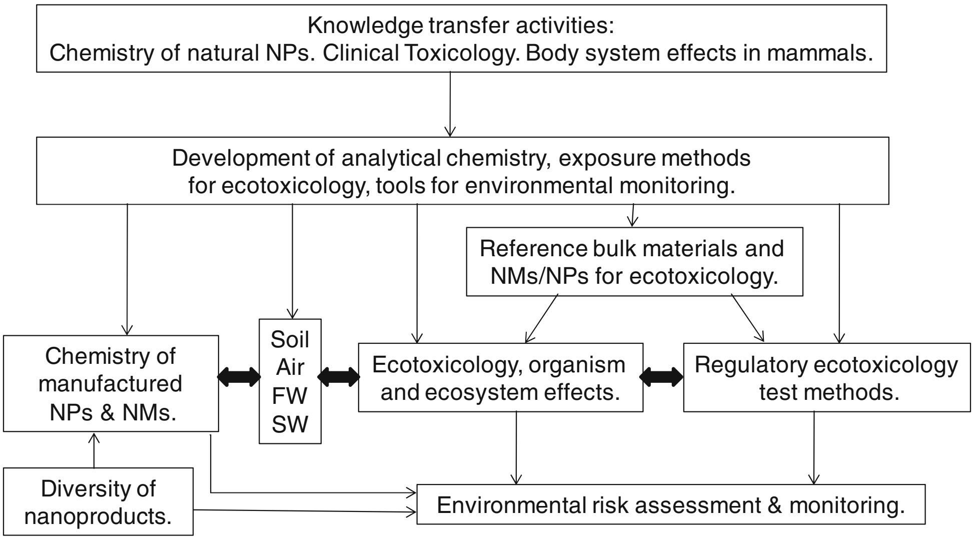

Fig. 1 Key challenges and knowledge gaps in the ecotoxicology of nanoparticles (NPs) and nanomaterials (NMs). Development of methods for analytical chemistry so that exposure mediums can be fully characterised before ecotoxicity experiments are conducted is critical. There is an opportunity for knowledge transfer of the physico-chemical techniques established for natural nanomaterials and colloids to manufactured NMs. Similarly, for the ecotoxicology, some indications of the types of toxic effects might be derived from studying the clinical or mammalian literature. There remain many challenges once the basic features of the physico-chemistry of a NP or NM, and an outline of the exposure method, have been established. These include differences in aggregation chemistry and behaviour of NP in different environmental matrices such as air, soils/sediments, freshwater (FW), and seawater (SW), with resultant differences in toxicity. There is also a need to perform ecotoxicological research on many different organisms across all the major phyla. The current literature shows over reliance on standard regulatory ecotoxicology test species and over reliance on FW organisms. Fundamental research should be used to inform and revise regulatory testing protocols that may require changes and additions to work well for NMs, perhaps including the use of new reference materials. All these aspects will need to be used to inform environmental risk assessments. At this early stage, some uncertainty in data interpretation seems likely given the need for method development in both the ecotoxicology and chemistry. Also, the large variety of nanoproducts, and lack of knowledge on adverse effects on a wide variety of wildlife, adds uncertainty about what products to prioritise for risk assess first; and whether or not the concept of protecting "most of the organisms most of the time" is achievable in the immediate future

ecotoxicologists compared to the properties of other pollutants like metals or pesticides. The application of these materials (i.e., nanotechnology) is also relatively new. Clearly, the scientific debate on the environmental safety of NMs needs to adopt a multi-disciplinary approach involving physicists, chemists, material scientists, biologists, toxicologists, risk assessors, regulators and policy makers. In order to have such a debate, the Society for Environmental Toxicology and Chemistry-UK branch (SETAC-UK) recently organised a meeting called the “2nd International Conference on the Environmental Effects of Nanoparticles and Nanomaterials” (hosted by the Natural History Museum in London) to bring this diverse group of professionals together. The articles in this volume of the Ecotoxicology journal are a synthesis of the main themes that emerged during the meeting, and for the first time, we bring the chemistry, biology, and risk assessment issues together in one volume. In this editorial, we aim to highlight the key themes, complexities, controversies and knowledge gaps on the ecotoxicology and environmental chemistry of manufactured NMs. In addition, we recognise that nature has been producing natural NPs and NMs for millions of years, and so we also aim to reflect on our knowledge of these natural materials and whether manufactured NMs represent a new or additional risk to the environment.

Natural nanomaterials: are organisms already adapted?

Nanoparticles are not a human invention and have existed naturally from the beginning of the Earth’s history. For example, NPs have been found in glacial ice cores some 10,000 years old (Murr et al. 2004), and there is evidence of natural NP formation in sediments at the Cretaceous-Tertiary (K-T) boundary (Verma et al. 2002). If we consider atmospheric dust alone, estimates indicate about one billion metric tons per year are produced globally (Kellogg and Griffin 2006), and even with a fraction of this as ultrafine particles, this would be millions of tons of natural nanoparticles. If we compare this against production estimates for manufactured NMs of the order of a few thousand tons per year for each major type of material (Borm et al. 2006), it is clear that exposure to natural NPs vastly out weighs any anthropogenic production. Table 1 gives some examples of natural substances that contain NPs. There are several mechanisms that create NPs in the environment and these can be either geological or biological. Geological mechanisms include physicochemical weathering, authigenesis/neoformation (e.g., in soils), and volcanic activity. These geological processes typically produce inorganic NPs. Biological mechanisms typically produce organic nanomolecules, although some organisms can produce mineral granules in cells (see below).

Table 1 Some examples of naturally occurring nanoparticles

Location of NPs	Particle types and ecotoxicological potential	Authors
Volcanic dust	Bismuth oxide nanoparticles were found in the stratosphere in 1985, and the presence of these materials was linked to volcanic eruptions in the 1980s	Rietmeijer and Mackinnon (1997)
Volcanic ash	Cristobalite (crystalline silica) extracted from volcanic ash from the Montserrat eruption causes lung inflammation and lymph node granuloma in laboratory rats	Lee and Richards (2004)
Ocean surface microlayer (SML)	Contains colloids, sub-micron components of phytoplankton, and carbon particles. Functions in transport of material at the air–water interphase. There are concerns that organic pollutants present in the SML may be adsorbed to colloids and other nano-scale material in the SML (Handy, unpublished)	El Nemr and Abd-Allah (2003) and Obernosterer et al. (2005)
Soil	A complex matrix containing mineral particles, colloids in pore water, and there are concerns about adsorption and binding of pollutants within the matrix	Reid et al. (2000)
Freshwater	Natural freshwater contain very complex colloid material which includes inorganic minerals and organic matter such as humic substances. Concerns exist over the accumulation and transportation of NPs in the colloid fraction	Lead and Wilkinson (2006)
Other natural waters	Nanoparticles were found in many types of natural water including the oceans, surface waters, groundwater, atmospheric water, and even treated drinking water. These include a wide variety of nanoscale mineral particles, and demonstrates the ubiquitous nature of nanoparticles in the natural environment	Wigginton et al. (2007)
Ice cores	Carbon nanotubes, carbon fullerenes and silicon dioxide nanocrystals have been found in 10,000 year old ice cores. The carbon nanoparticles are assumed to be derived from natural combustion processes and deposited into the ice core via atmospheric deposition	Murr et al. (2004)
Historic sediments	Examination of the Cretaceous-Tertiary (K-T) boundary layer at Gubbio, Italy showed iron particles (hematite) and silicates. The average particle size of the hematite was 16–27 nm. There is speculation that meteorite impacts could alter NP formation in sediments at the K-T boundary	Verma et al. (2002)

A brief explanation of the geological processes follows (for a general introduction to geological processes see Lutgens et al. 2005):

(i) Weathering is the result of physical (abrasion) or chemical (dissolution) decomposition of rock material, to produce a powder. Part of this powder will naturally exist as NP, either as a primary effect of the decomposition, or through further physical/chemical weathering. Weathering has been active on Earth for the best part of the past 4 billion years, and ever since the Earth's surface was cold enough for water and an atmosphere to exist.

(ii) Authigenesis/neoformation is the reverse of the previous process. It takes place when chemical degradation eventually results in high enough concentrations of certain dissolved species to exceed the saturation in solution of a phase, leading to its nucleation and growth. The early forming nuclei of authigenic or neoformed phases are sub-nanometric in size and may either re-dissolve, grow to form larger particles, or remain nanosized. The nanoscale particles can be stabilised in solution by organic species such as humic substances (Lead and Wilkinson 2006), or simply because it is not thermodynamically possible to grow larger particles. Many common soil and water components contain natural NPs that are grown by authigenesis/neoformation including clay minerals and iron oxyhydroxides. Nanoparticles created by ocean spray can be classified under authigenesis.

(iii) Volcanic eruptions, including also geysers and other geothermal/hydrothermal activities produce a variety of particle sizes, which include NPs. The latter stay airborne longer and travel further (see Table 1). Meteorite impacts may also result in NP formation.

Many biological processes typically operate at the nanoscale and many biological entities, from proteins/peptides, DNA/RNA, ATP, to viruses are nanosized. Some of these are clearly released into the environment directly from the organism (e.g., mucoprotein exudates from algae and animals, dispersion of virus particles), and in addition may also be released during the degradation of biological matter in the environment (e.g., humic and fulvic acids). The latter are typically 50–200 nm in size, occur freely in natural waters and soils, and are particularly chemically active (e.g., Lead and Wilkinson 2006).

Given these many forms of natural NPs in the environment, and within the biological structures of organisms, one would assume that organisms have evolved and adapted to living with these materials. However, the question of whether organisms are adapted to natural NPs is a function of exposure, dose, and the speed of change in habitat conditions. For example, while we accept that the lung is adapted to clearing small amounts of airborne particles, lung function would be compromised in a more dramatic environmental event (e.g., dust storm or volcanic eruption). Similarly in the aquatic environment, for organisms that normally live in clear water, a storm event that creates high levels of suspended solids and turbidity may be a challenge. Alternatively, animals that normally live in naturally turbid waters (e.g., many estuaries) may already be adapted, and may not find the turbidity a new challenge.

Organisms on Earth have therefore clearly been exposed to nano-sized materials throughout evolution, and indeed are made of nano-sized components, so why are manufactured NPs a cause concern? Firstly, because naturally occurring NPs, under certain circumstances, can be toxic to life forms; and so this raises concerns that manufactured NPs could also be toxic. An example of toxicity induced by a natural source of particles is volcanic dust, and it has been shown that toxicity is exacerbated by small particle size (Lee and Richards 2004). A further cause for concern is that many natural NPs are transient in the environment, often disappearing through dissolution, or becoming larger through particle growth or aggregation. Alternatively, some manufactured NPs may persist because they can be stabilised by capping or fixing agents such as surfactants or organic matter (Handy et al. 2008). Of course, manufactured NPs may contain chemically toxic components in concentrations or structural forms that do not occur naturally, but we must not exclude the possibility that natural NPs grown in polluted environments could also inadvertently incorporate toxic material. The issue of structural form and shape may be as critical as the chemical reactivity. In nature of course, there are a myriad of possible structures, including crystal structures to minerals, types of clay deposits and colloidal organic matter, but the question remains as to whether manufactured NMs present new structures that are not found in nature, and are also toxic. There is also incidental production of NPs from human activity (e.g., wear of car tyres, urban air pollution) that may also present a toxicological risk (reviewed in Handy and Shaw 2007).

Physico-chemistry of nanoparticles

The behaviour of manufactured NPs in environmental matrices such as natural waters, sediments or soils is likely to be complex and involve several processes (Elimelech et al. 1995; Buffle and van Leuween 1992; Grasso et al. 2002; Lead and Wilkinson 2006; Christian et al. this volume) that may also influence ecotoxicity (Handy et al. 2008). These include:

(i) Aggregation chemistry and the ability of manufactured NPs to form stable dispersions in liquids such as water.

(ii) The effects of particle shape, size, surface area and surface charge on aggregation chemistry and ecotoxicity.
(iii) Adsorption of manufactured NPs on to surfaces, including the exterior surfaces of organisms.
(iv) The effect of other abiotic factors on all the above including the influence of changing environmental pH, salinity (or ionic strength), water hardness, and the presence of natural organic matter.

Ecotoxicologists often need to handle test materials in some kind of liquid or solution phase in order to perform exposures or administer a dose. However, NPs do not necessarily *dissolve* in solution, but may form a colloid dispersion. The phrase "colloid" applies to particle sizes or suspensions of material in the 1 nm–1 μm size range (Lead and Wilkinson 2006). In colloid chemistry, particles may remain dispersed, or alternatively, aggregation processes may remove the material from the liquid phase. In practise, even apparently stable dispersions will gradually aggregate out of the aqueous phase over time (e.g., over several days). The rate of aggregation of particles in an aqueous medium will partly depend on particle–particle collision frequency (e.g., Brownian motion and particle number concentration in the medium), the energy of the collision, and the attractive–repulsive properties of the particles involved (e.g., repelling surface charges on two positively charged particles); as well as similar interactions with other colloid materials such as natural organic matter present in the medium (review, Handy et al. 2008). After an initial collision, particles may remain in aqueous phase as single particles, or form particle–particle, particle–cluster and cluster–cluster aggregates. The forces involved in the collisions include Borne repulsion, diffuse double layer potential, and van der Waals attraction. These forces are described by the extended DLVO theory developed by Derjaguin and Landau (1941), and Verwey and Overbeck (1948), hence the name DLVO. Aggregation phenomena have practical implications, such as the attachment of particles to the walls of experimental equipment (glassware, fish tanks, scientific instruments), and the tendency of NPs to aggregate in some natural waters, or on the organisms themselves (Smith et al. 2007). There will also be toxicological implications regarding fate and behaviour of the materials, and the types of ecosystems and organisms exposed (see below).

However, DLVO theory does not account for the effects of particle shape, charge heterogeneity, or surface roughness (Elimelech et al. 1995; Grasso et al. 2002). Surface charge and shape effects are relevant to the fate and behaviour of manufactured NPs in the environment, and therefore to the exposure of organisms. For example, additions of salt to the medium (increasing ionic strength) may provide charge shielding and/or compress the charge layer on the surface of the NP (the electric double layer or EDL) so that the particle collisions leads to attachments of particles and therefore aggregation. Similar arguments might be applied to charge screening of negatively charged surfaces by additions of acid. Thus aggregation in seawater is more likely than in freshwater, and the pH of the water may also influence the aggregation rate depending on the surface charge of the particles involved. Alternatively, additions of natural organic matter could coat the surface of NPs, resulting in NPs that tend to stay dispersed rather than aggregate. For example, additions of negatively charged humic and fulvic acids to positively charged mineral NPs in natural freshwater. Surface charge effects could also be influenced by the presence of competing cations like Ca^{2+} that might screen a negatively charged surface. Thus NP dispersion will also depend on the type and amount of natural organic matter in the water and the free cation concentration (Hyung et al. 2007, Giasuddin et al. 2007).

Particle interactions will also be influenced by particle shape, not only because diffusion rates of the material will change with the aspect ratio of the material (e.g., higher drag on a tubular structure compared to a perfect sphere), but also because of steric hindrance in the collisions. The shape may make it difficult for particles to approach each other. Some of these properties might be overcome by adding detergents or surfactants that could coat the particles and/or change their shape or surface charge. For example, Smith et al. (2007) used sodium dodecyl sulphate, SDS, detergent to disperse single walled carbon nanotubes (SWCNT). Alternatively, the presence of other organic matter may help promote polymer bridging between the NPs and organic matter so that aggregation is promoted (Wilkinson et al. 1997).

Aggregation may result in materials being deposited on organisms in the aquatic environment (e.g., aggregation of material from the water on to benthic organisms). Alternatively, NPs could be adsorbed to the exterior surface of the organism. The idea of surface acting toxins/toxicants is not new to ecotoxicology (Handy and Eddy 1991). The surface of the organism may present a complex unstirred layer (USL), with polyanionic mucus secretions, and ligands on the cell surface (e.g., gill epithelial cells) which are also predominantly anionic (review Handy and Eddy 2004). USL formation at the organism surface could result in shear forces that either cause particle aggregation (peri-kinetic aggregation, Handy et al. 2008) or result in higher concentration of the NPs at the surface of the organism compared to the bulk water due to the slow flow or the viscous properties of the USL. Such processes have already been implicated in TiO_2 NP toxicity to trout (Federici et al. 2007). Similar adsorption phenomena might also apply to solid–liquid interfaces, and air–water interfaces in the environment; resulting in high NP concentrations at these interfaces. For example, in marine surface waters (the surface microlayer, Wurl and Obbard 2004) the viscous properties of solution will be a dominant force in the diffusion of NPs (i.e., a low Reynolds number). This could result in the trapping of high concentrations of NPs in the ocean surface microlayer compared to the surrounding seawater. This has been a concern for micron scale particulates and exposure of marine invertebrates (Simpkiss 1990). Organisms in this ocean surface microlayer such as eggs, and early life stages such as zooplankton, may therefore be particularly vulnerable. Similar arguments could be applied to organism in biofilms and at sediment surfaces.

The physico-chemistry above highlights a number of key issues for the ecotoxicologist. These include:

(i) The fate and behaviour of most manufactured NPs are likely to be different in seawater compared to freshwater, and therefore ecotoxicity may also be different.
(ii) Ecotoxicity in freshwater may be particularly influenced by the presence of organic matter, changes in pH, and the presence of cations such as Ca^{2+}.
(iii) Environmental exposure will not be homogeneous. Adsorption and aggregation phenomena may promote local high concentrations of manufactured NPs in sediments, on biofilms, or in microsurface layers (e.g., ocean microsurface layer).
(iv) Adsorption phenomena may drive surface acting toxicity to organisms, and this could occur without appreciable bioaccumulation within the organisms.
(v) Uptake into the organism will depend on the aggregation chemistry on the exterior surfaces of the organism, and the behaviour of manufactured NPs in body fluids such as plasma.
(vi) Toxicity may be a function of particle size and shape, and that there may be inherent differences in the toxicity of NPs compared to micron scale particles.

In addition, it is also worth considering how the above chemistry will impact on the behaviour of NPs in polluted environments, and on whether NPs are likely to interact with other (non-nano) pollutants in the environment. In theory, the presence of surfactants in polluted environments might stabilise manufactured NPs in the aqueous phase (as they would for natural NPs above). Alternatively, some types of organic matter and particulate materials in effluents might cause NP aggregation. It is also possible that manufactured NPs could adsorb organic chemicals to the outer surface of the particle. This might reduce the bioavailability of the chemical, or alternatively, the NP may act as a delivery vehicle for the organic chemical. Baun et al. (2008) recently showed that the uptake of phenanthrene by *Daphnia magna* was much faster in the presence of C_{60} NPs and was probably due to the NPs enabling delivery of the phenanthrene to the test organism. Clearly, interactions of manufactured NPs with existing pollutants that exacerbate toxicity is a concern.

Biological effects

The literature on the ecotoxicity of NPs is still emerging, and there have been several recent reviews on the ecotoxicity of manufactured NPs and NMs (e.g., Oberdörster et al. 2006; Crane and Handy 2007; Moore 2006). At this early stage most ecotoxicological studies have been observational or "proof of principle" experiments that have tried to document toxic effects, and the concentrations of NPs that produce these effects in different groups of organisms. In this volume of the Ecotoxicology journal we attempt to summarise what is known about ecotoxicity to different groups of organisms. The papers in this volume are therefore arranged with phylogeny in mind with reviews on algae and plants (Navarro et al. this volume), bacteria and other microbes (Neal et al. this volume), invertebrates (Baun et al. this volume), as well as fish and considerations for lower vertebrates (Handy et al. this volume).

The literature on mammalian models has recently been reviewed in the context of the environment and routes of human exposure to manufactured NPs (Handy and Shaw 2007). These mammalian reports have focused on respiratory toxicology and inflammation reactions to NP exposure (e.g., Oberdörster et al. 1992; Burmudez et al. 2004; Lam et al. 2004). Handy et al. (2008) point out that the mammalian lung epithelium has some relevance to ecotoxicology because the lung is representative of a typical mucous epithelial tissue and is not fundamentally different in structure to other epithelia such as the gills or guts of aquatic organisms, or the body surface of earth worms. Fish gills are certainly sensitive to some manufactured NPs (TiO_2 NPs, Federici et al. 2007; single walled carbon nanotubes, SWCNT, Smith et al. 2007). Furthermore, the inflammation and immune responses to particulate exposures in mammals raise concerns about immunity and the long term health of other organisms exposed to NPs. Mammalian studies also confirm the notion from the chemistry above that particle size, shape, surface properties may be important factors in the uptake and toxicity of NPs (Maynard and Aitken 2007).

There are many gaps in our knowledge on the ecotoxicity of NPs. Most of the available acute toxicity data are on freshwater species, and mainly on species used for regulatory toxicology (e.g., *D. magna*, Lovern and Klaper 2006; fathead minnows, *Pimephales promelas*, Zhu et al. 2006). Most sub-lethal studies on vertebrates are on freshwater fish, with studies on C_{60} fullerenes in large mouth bass (*Micropterus salmoides*, Oberdörster 2004), fathead minnow (*P. promelas*, Oberdörster et al. 2006; Zhu et al. 2006), and Japanese medaka (*Oryzias latipes*, Oberdörster et al. 2006). The sub-lethal effects of CNT's (Smith et al. 2007) and TiO_2 NPs (Federici et al. 2007) on rainbow trout (*Oncorhynchus mykiss*) have also been studied. Sub-lethal and chronic studies on invertebrates species are, so far, mainly on freshwater animals. Notably, studies on C_{60} in *D. magna* and *Hyallela azteca*, which explored effects on mobility, moulting and feeding behaviour (Oberdörster et al. 2006). More recently, effects on the locomotor behaviours of invertebrates have been reported (e.g., Lovern et al. 2007). Sub-lethal effects at the cellular level such as biochemical change and genotoxicity also require investigation in wildlife (Vevers and Jha this volume).

Clearly, more studies are needed on marine and terrestrial invertebrate species, as well as other vertebrates including amphibians, reptiles and birds. Data on terrestrial plants and other photosynthetic organisms are also lacking (Navarro et al. this volume). However, high concentrations of NPs have been shown to inhibit the growth of algae (e.g., EC_{50} of around 44 mg l^{-1} for *Desmodesmus subspicatus* exposed to titanium NPs, Hund-Rinke and Simon 2006) and NP aggregates can form on the cell walls (e.g., on marine phytoplankton, Scarano and Morelli 2003). Similarly, knowledge of the ecotoxicology of NPs to bacteria and other microbes is limited, even though some manufactured NPs have been designed as cleaning agents with antibacterial properties (e.g., titanium and silver NPs, Fu et al. 2005; Duran et al. 2007). Clearly, one concern is that some manufactured NPs may also be toxic to microbes in the environment. At least one experiment with C_{60} fullerenes (Tong et al. 2007) suggested little impact on the microbial community in soil after a 30-day exposure to 1 mg C_{60} g^{-1} of soil. In contrast, C_{60} in suspension has been shown to have toxic effects on bacterial cultures in the laboratory. Lyon et al. (2005) report minimum inhibitory concentrations of C_{60} of 0.5–1.0 and 1.5–3.0 mg l^{-1}, respectively, for *Escherichia coli* and *Bacillus subtilis* growth. This difference illustrates the importance of the environmental matrix in the toxicity of NPs, toxicity in laboratory solutions may be somewhat different from that in real environmental samples. However, this is a familiar problem for many pollutants, and once the proof of concept and observational studies are complete in the laboratory, we need to explore environmentally realistic exposure scenarios and more complex matrices. These would include studies with different routes of exposure (e.g., diet versus water) and chronic exposure experiments which are generally lacking at present. Development of methodologies to measure NPs in complex environmental matrices (soil, sediments, natural waters) will be an urgent and essential prerequisite to environmentally realistic studies of NP ecotoxicity.

To date, ecotoxicological studies have not been performed to the level of detail that would enable a mechanistic analysis of absorption, distribution, metabolism and excretion (ADME). The first step in the biological uptake of any substance is the adsorption of the material onto the exterior surface of the organism (Handy and Eddy 2004), and the physico-chemistry above predicts aggregation or precipitation of NPs on to the exterior surface of organisms. This notion is supported by experimental observations, for example, the aggregation of SWCNT on the gill mucus of trout (Smith et al. 2007). The next step is uptake across the cell membrane and the mechanisms involved remain to be investigated. These could include the dissolution of metal NPs and aqueous uptake of the metal ions on transporters in the membrane, diffusion of hydrophobic NPs across the cell membrane (e.g., in a similar way to lipid micelles in the gut), or endocytosis of the primary particles or aggregates. It seems unlikely that NPs could be moved across the cell membrane via ion transporters because the particles are much bigger than ions, and presumably would not fit the binding sites. For diffusional uptake, then the octanol–water partition coefficients for the ligands on the exterior surface of the NP (i.e., not the chemistry in the core of the NP) might partly reflect the risk of diffusive uptake. Moore (2006) argues the case for endocytosis and points out that marine bivalves such as *Mytilus edulis* might take up NPs using endocytosis, and demonstrated that polyester NPs were taken up into endosomes and lysosomes of mussels. This mode of uptake may be especially relevant where aggregation of NPs on the surfaces of the organism occur. Moore (2006) also raised concerns about NPs acting as delivery vehicles for other chemicals via endocytosis pathways.

Distribution within the body, and the target organs for NPs, are mostly unknown. These processes will partly depend on the solution chemistry of the body fluids, as well as the behaviour of NPs in those fluids. Blood plasma is a saline solution that would be expected to cause aggregation of NPs. Alternatively, NPs could be carried on albumins or other proteins that are typically found in body fluids. These are not new problems, for example the bulk transport of organic chemicals on lipoproteins in the blood are well known (Noren et al. 1999), but we do need to describe these behaviours in the body fluids of different organisms for NPs. Measurement of NP concentrations and identifying NPs in tissues may confirm the target organs within an organism. However, the current techniques for doing this are labour intensive. Particles can be identified in tissues using the electron microscope (see discussion in Handy et al. 2008), but the sample preparation takes time, and there is always the risk of introducing aggregation artefacts during processing of the material. For metal NPs, in addition to electron microscopy, it may be possible to measure metal concentrations in the tissues by inductively coupled plasma mass spectrometry (ICP-MS) or similar techniques that involve the analysis of an acid digest of the tissue. This approach may work well for unusual materials that are not normally found at high concentration in organisms (e.g., gold), but it may be more difficult to pick the metal NP effect from the background levels of metal already in the organism (e.g., micromolar amounts of Ti metal in fish tissues, Federici et al. 2007). Furthermore, tissue digests may not allow any determination of particle size distribution in the organ. Clearly, these approaches are best employed for fish, large crustaceans or other organisms where it is possible to dissect out the organs for analysis. However, we should also develop in situ methods to look at body distributions in small invertebrates.

The target organs may also reflect the biology or life style of the organism. For example, crustaceans are well known for their ability to sequester toxic metals in granules in the hepatopancreas and other tissues (e.g., Barka et al. 2007). It might therefore be possible for crustacea to do the same with metal NPs, and this would make the organisms potent bioaccumulators of NPs. Alternatively, processing of nanoscale granules may be different. Excretion of NPs by organisms might simply occur by reversal of the uptake process (e.g., exocytosis instead of endocytosis), or require specific methods of excretion. NP measurements have yet to be made in the urine, bile or faecal waste of wildlife.

Ecotoxicity test methods and environmental risk assessment

Crane and Handy (2007) recently reported to the Department for the Environment, Food and Rural Affairs (DEFRA) in the UK on the possible issues around regulatory ecotoxicity testing and test methodology for NMs. The key issues for the ecotoxicologist are summarised (Crane et al. this volume). Several main issues have emerged including:

(i) Methods of dispersion and whether or not ecotoxicity tests should use dispersed NPs.
(ii) The chemical characterisation of the test material.
(iii) Reference NMs for regulatory ecotoxicology.
(iv) Modifications to test methods or solution preparation that enable existing regulatory ecotoxicity tests to work with NMs.
(v) Triggers for conducting the tests, and whether or not we need new tests, or additional measurements within existing tests, to quantify novel or unusual toxicological properties.

Most of the manufactured NPs used in ecotoxicological studies so far, have not been designed to disperse readily in water. For example, carbon nanotubes are almost impossible to disperse in water by physical methods such as sonication or stirring alone, and may require the use of a dispersing agent (e.g., Smith et al. 2007). Furthermore, the chemistry above suggests that NPs will aggregate in many types of natural waters (e.g., hard freshwater and seawater), and it could be argued that it is more ecologically relevant to use the natural aggregated NPs for regulatory testing. However, if the purpose of the experiment is more fundamental, such as investigations of toxic mechanisms, then it may be useful to compare the effects of dispersions with aggregated material.

Chemical characterisation of the test material is also critical (Crane and Handy 2007). There is an opportunity in this new area of ecotoxicology to set the standards for chemical characterisation of NPs for fundamental research, as well as for regulatory toxicity testing. Information on particle size distributions, surface charge, surface area or reactivity, impurities in the NMs etc is sought. Currently, there are gaps in our analytical capability for describing NMs on a routine basis for ecotoxicology (Hassellov et al. this volume), but having detailed information on physico-chemical characteristics would enable accurate comparisons between data sets from different laboratories, or on different species with the same materials. Furthermore, it would enable retrospective sorting of data sets for particle size effects, surface area effects, confounding effects of solvents or impurities etc. Particle size and surface area may be particularly important, as mass concentration (e.g., mg l^{-1}) may not be the best metric to describe the dose–response relationship (Oberdörster et al. 2007).

There is also some controversy over the use of reference materials. In mammalian respiratory toxicology, reference particles such as carbon black, quartz or silica have been used as positive controls in experiments (Aitken et al. 2007). Federici et al. (2007) point out that many of these materials may not be toxic to aquatic species. For example, many organisms live in sand substrates and it seems unlikely that these particulate materials would be toxic. Reference materials are discussed (Crane et al. this volume) and the question remains as to what materials should be selected as references for ecotoxicology, and whether or not these are needed in all experiments.

A number of key issues are also highlighted for ecological risk assessment. There are many types of NMs and one of the challenges is to categorise and prioritise these materials for the purposes of ecotoxicological risk assessments (Owen and Handy 2007), for product life cycle analysis, and the potential points in the product life cycle where these materials may enter the environment (Foss-Hansen et al. this volume). We also need to develop techniques and methods to measure manufactured NP in the environment, although risk predictions suggested low μg l^{-1} concentration of some NPs in the environment (Boxall et al. 2007). The consensus view from the scientific community seems to be that a tiered ecological risk assessment can be applied to NMs and that some sensible linkage should remain with ecologically relevant end points such as population growth and reproduction (Crane and Handy 2007). So for existing chemicals effort should focus on the differences between the NM and bulk materials of the same chemical (e.g., fullerenes compared with graphite). If evidence suggests the nanoscale material has different toxicological properties then this might warrant a full series of ecotoxicity tests and a risk assessment. Relevant reference materials and standards would also need to be established for ecotoxicological testing (Crane and Handy 2007). Clearly, any new material (nano or otherwise) should be subject to environmental risk assessment under frameworks such as REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals). Although there are concerns that REACH only applies to substances with an annual production in excess of 1 tonne and that not all NMs will reach this trigger level (Handy and Shaw 2007).

In conclusion, the scientific community has started to report data on the ecotoxicity of some NPs and NMs. The challenges include broadening the data set so that ecotoxicity is assessed on organisms from different phyla. We should recognise that the behaviour of NPs in the marine environment is likely to be very different from some freshwaters, and that a fundamental understanding of natural NPs and colloids may be a prerequisite to elucidating the fate and behaviour of manufactured NPs in complex environmental matrices. Ecotoxicologists therefore need to learn some physico-chemistry, and work more closely with physicists, chemists, and material scientists to achieve the correct interpretation of data from ecotoxicity experiments. There is much method development and validation to be done for both the chemistry and the biology, and it is essential that these proceed in parallel. We should not wait for the chemistry to be perfected before studying biological effects, but equally, there should be some key chemical characterisation in every experiment so a more realistic interpretation of the results is achieved. All these facets will need to feed into the environmental risk assessment process. The diversity of nanoproducts and current lack of experimental data on many species, suggests the scientific community is currently some way off from being able to show protection of most of the organisms most of the time in the natural environment.

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